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Preliminary communication

A rapid, convenient method for the determination of hexosamines as *O*-acetylated-*O*-methyloximes by gas–liquid chromatography*

THOMAS P. MAWHINNEY, MILTON S. FEATHER,
Department of Biochemistry, University of Missouri, Columbia, Missouri 65211 (U.S.A.)
J. RICARDO MARTINEZ and GUILIO J. BARBERO
Department of Child Health, University of Missouri, Columbia, Missouri 65211 (U.S.A.)
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Over the past years, a number[1–3] of procedures have been reported for producing volatile derivatives of sugars suitable for use in g.l.c. analyses. Those derivatives most widely used are the alditol acetates[2] and the aldononitrile acetates[3,4]. None of these methods are generally useful for analysis of 2-amino-2-deoxyhexoses because of lengthy derivatization times, irreversible absorption to column supports, and excessively long retention-times. It is noteworthy that Schwarzmann and Jeanloz[5], and Stellner[6] and co-workers, have reported that partially acetylated, partially methylated 2-amino-2-deoxy-D-glucitols may be chromatographed and that the mass spectra of such derivatives are useful as structural tools. Neither of the reports, however, evaluated these derivatives for routine quantitative analysis.

Laine and Sweeley[7,8] have reported on the use of *O*-trimethylsilyl, *O*-methyloxime derivatives of neutral sugars for analysis by both g.l.c. and m.s. Although some instances of multiple peaks were observed (separation of *anti* and *syn* isomers), the derivatives appear to be formed rapidly and completely.

This communication describes the conversion of three amino sugars into their *O*-acetylated, *O*-methyloximes and the use of these derivatives for g.l.c. analysis. The procedure permits formation of the derivatives in < 40 min, and shows by using most of the commonly available columns, retention times of < 15 min, thus providing a rapid and accurate method for the detection and analysis of amino sugars. The sugars used were the hydrochlorides of 2-amino-2-deoxy-D-glucose (GlcN), 2-amino-2-deoxy-D-mannose (ManN), and 2-amino-2-deoxy-D-galactose (GalN).

The procedure for derivatization is as follows: the *O*-methyloximation reagent is a solution of *O*-methylhydroxylamine hydrochloride (300 mg) in anhydrous methanol (1.0 mL) and anhydrous pyridine (1.78 mL), to which is added 0.22 mL of 1-dimethylamino-2-propanol. The sample (1.0–5.0 mg of sugars) is placed in a 1.0-mL, screw-capped

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vial and ~20 μL of 1 : 16 (v/v) piperidine–methanol is added, and the suspension is stirred for 5 min. The presence of water is not detrimental to the reaction. Sugars (1–5 mg) may be dissolved in up to 20 μL of water prior to derivatization. In this situation, the piperidine–methanol solution is omitted from the procedure. The oximation reagent (0.1 mL) is added, and the vial is heated for 15 min at 70°, after which time the methanol is evaporated from the solution by directing a stream of dry air into the opened vial for 2 min. At this time, 1 : 3 (v/v) pyridine–acetic anhydride (1.0 mL) is added and the vial is again heated for a further 25 min at 70°. The solution is then evaporated to near dryness with a stream of dry air, 1 mL of chloroform is added, and the chloroform is extracted by layering 1.0 mL of M hydrochloric acid over the organic phase; the mixture is shaken and then extraction is repeated, in the same manner, 3 more times with water. The chloroform phase, which contains the derivative, may then be used directly for chromatographic injection, be diluted, or be evaporated and reconstituted with a different organic solvent. Methyl α-D-glucopyranoside may be used as an internal standard.

A number of different liquid and solid supports were used in the chromatographic testing, including 1 or 2% diethyleneglycol adipate on Chromsorb W-HP (100–120 mesh), 3% OV-225 on Supelcoport (80–100 mesh), 1% ECNSS-M on Chromsorb W-HP (100–120 mesh), and Silar 7CP on Chromsorb W-HP (100–120 mesh).

In all instances, the sugar derivative emerged as a single, symmetrical peak with no obvious separation of *anti* and *syn* isomers. As evidenced by peak areas, formation of the *O*-methyloxime was complete in < 10 min and the subsequent acetylation also required less than 15 min. On all columns tested, GalN and ManN showed some peak overlap, but were readily separated from GlcN. Optimal conditions for separation of the D-manno and D-galacto isomers involved a slightly different program-sequence than that used for a mixture of GlcN and either of the other two sugars. Typical separations are shown in Figs. 1A and 1B. The derivatives, in chloroform, appear to be quite stable and may be stored for several months without apparent decomposition.

To examine whether both *anti* and *syn* isomers are produced during the *O*-methyloximation procedure, the derivative from GlcN was subjected to preparative t.l.c. on silica gel with 5 : 10 : 8 : 2 petroleum ether–chloroform–ethyl acetate–methanol as irrigant. The two bands that appeared on the plates were isolated and subjected to g.l.c. and each component was found to have the same retention-time. It thus appears that both *anti* and *syn* isomers are formed, but they are not resolved under the g.l.c. conditions. Derivatization of both ManN and GalN, followed by examination by t.l.c., showed a similar 2-band chromatogram.

It is also noteworthy that the derivative of GlcN, when subjected to mass spectrometry, showed a molecular ion (*m/e* 418) having a relative abundance of 28%, a surprisingly high figure for a carbohydrate derivative. Other significant peaks observed were *m/e* (%): 130 (197), 187 (86), and 289 (31).

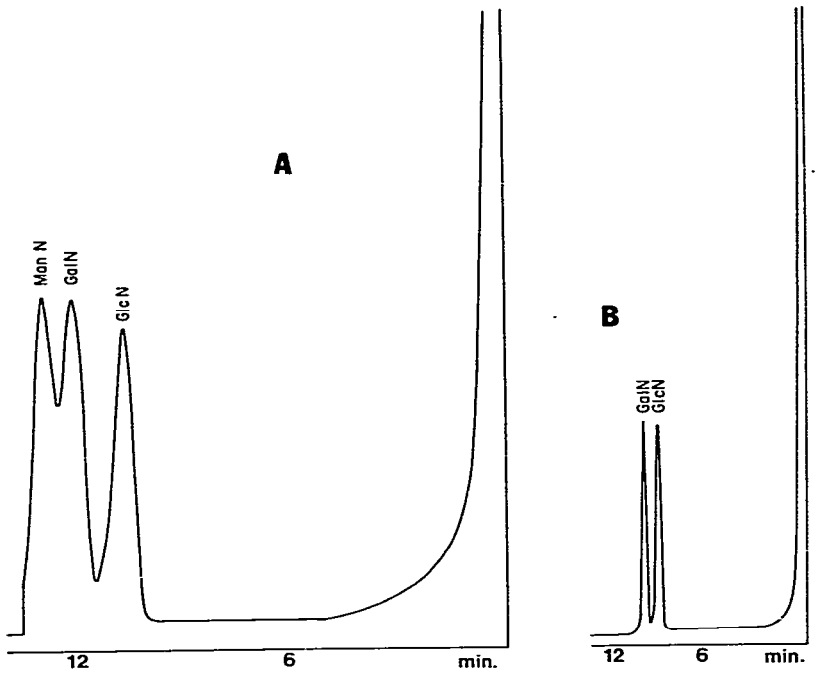

Fig. 1. Separations of (A) GlcN, GalN, and ManN, and (B) GlcN and GalN on a 1/8-in nickel column containing 1% diethyleneglycol adipate on Chromosorb W-HP, with nitrogen as carrier gas at a flow rate of 24 mL per min. For A, the following conditions were used: initial temperature, 190 (1-min hold) to 205° at 1° per min. Retention times for ManN, GalN, and GlcN were 11.5, 11.0, and 10.0 min, respectively. For B, the following conditions were used: initial temperature, 215 (5-min hold) to 235° at 5° per min. Retention times for GalN and GlcN were 10.0 and 11.0 min, respectively.

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